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The Effects of Clobazam and Diazepam on Central Catecholaminergic System

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SUMMARY

The effects of the 1,5-benzodiazepine clobazam and diazepam were evaluated with regard to behavioral locomotor changes in rats. Diazepam was more active than clobazam in reducing locomotor activity. The stimulation of locomotor activity induced by L-DOPA plus benserazide, and methamphetamine was significantly counteracted by diazepam but unaffected by clobazam. Hypomotility produced by α-methyl-p-tyrosine was markedly augmented with both drugs. It is concluded that both benzodiazepines may exert their behavioral depressant effects by reducing catecholaminergic activity. Diazepam is more effective than clobazam in reducing catecholaminergic activity.

INTRODUCTION

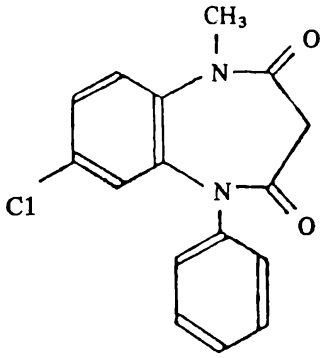
Despite their widespread popularity in the treatment of psychiatric and seizure disorders, the mechanisms whereby benzodiazepines interact with central neurons have not yet been clearly defined[1]. Several investigators have suggested that benzodiazepines decreased the turnover of brain catecholamines and 5-hydroxytryptamine (5-HT) in the central nervous system, and enhanced synaptic transmission by r-aminobutyric acid[2,3]. Other effects, however, e.g. the anxiety-reducing action, might be due to changes in monoaminergic systems secondary to altered GABA ergic transmission[3,4]. Benzodiazepines are used as anticonvulsants, muscle relaxants, sedatives and anxiolytic agents, and hypnotics, however, the mechanism of their action is still controversial.

Clobazam (1-phenyl-5-methyl-8-chloro-1,2,4,5-tetra-hydro-2,4-diketo-3H-1,5-benzodiazepine, Hoechst, Japan) (Fig. 1), is a new 1,5-benzodiazepine derivative. The structure of this compound differs from that of conventional minor tranquilizers, since the nitrogen atoms in the heterocyclic ring are in the 1,5-instead of the 1,4-position. Pharmacologically, the anticonvulsant effect of clobazam was comparable to that of diazepam[5], but clobazam was less active in Geller conflict in the rat[6] and inhibiting polysynaptic spinal reflexes in the rabbit[7]. However, effects of clobazam on the central nervous system remain obscure. Therefore, the present study was designed to investigate the effects of acute clobazam on central catecholaminergic system affecting locomotor activity in the rat. Furthermore, some of the locomotor activity of clobazam were compared with those exerted by a widely used tranquilizer, diazepam.

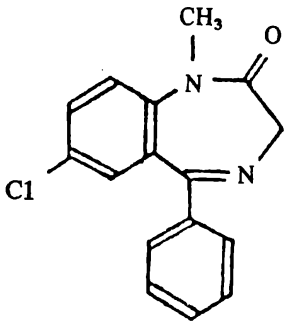
MATERIALS AND METHODS

1. Animals

Male Sprague-Dawley rats, weighing between 195 to 230 g, were used throughout these studies.



1-phenyl-5-methyl-8-chloro-1,2,4,5-tetrahydro-2,4-diketo-3H-1,5-benzodiazepine (clobazam)



DIAZEPAM (DZP)

Fig. 1 The chemical structures of clobazam (CBZ) and diazepam (DZP)

2. Drugs and treatment regimens

The following compounds were used in these experiments. Clobazam (CBZ, Hoechst Japan), diazepam (DZP, Hoffmann-La Roche), methamphetamine HCl (MAPT, Dainippon pharmaceuticals), L-DOPA (Kyowa Hakko Kogyo Co., Japan), benserazide HCl (Hoffmann-La Roche), α-methyl-p-tyrosine methylester HCl (α-MT, Sigma).

Clobazam or diazepam 1 and 10 mg/kg I.P. were administered in a fixed dosage volume of 2 ml/kg body weight. MAPT was given S.C. into the dorsal part of the neck in a volume of 1 ml/kg, other compounds were injected I.P. in a volume of 2.5 ml/kg. They were given before clobazam and diazepam as follows: α-MT 100 mg/kg 2 hr, L-DOPA 200 mg/kg 50 min., benserazide 50 mg/kg 80 min. Control rats received injection of the appropriate vehicle 0.5% CMC. Clobazam, diazepam, L-DOPA were suspended in 0.5% CMC solution, all the other compounds were dissolved in purified water. L-DOPA was prepared immediately before use.

3. Locomotor activity measurements

The locomotor activity of individual rats was measured with an Animex Activity Meter Type S, and an Animex Counter Type 1-6 (Farad Electronics, Hägersten, Sweden). The sensitivity and tuning of the instrument was adjusted to 35 μA to enable all kinds of motor behavior, including locomotion, rearing, grooming, and sniffing to be jointly recorded. Activity was recorded for 60 min intervals for 3 hr, starting 5 min after inserting the animals into the test cage. Each rat was used only once.

4. Statistical Analysis

Results were analysed statistically using Student's-t-test to compare intergroup differences.

RESULTS

1. The effects of clobazam and diazepam on locomotor activity

As shown in Figure 2, the lower dose of clobazam, 1 mg/kg, had no statistically significant effect on locomotor activity ($p>0.05$), while the higher dose of 10 mg/kg significantly decreased

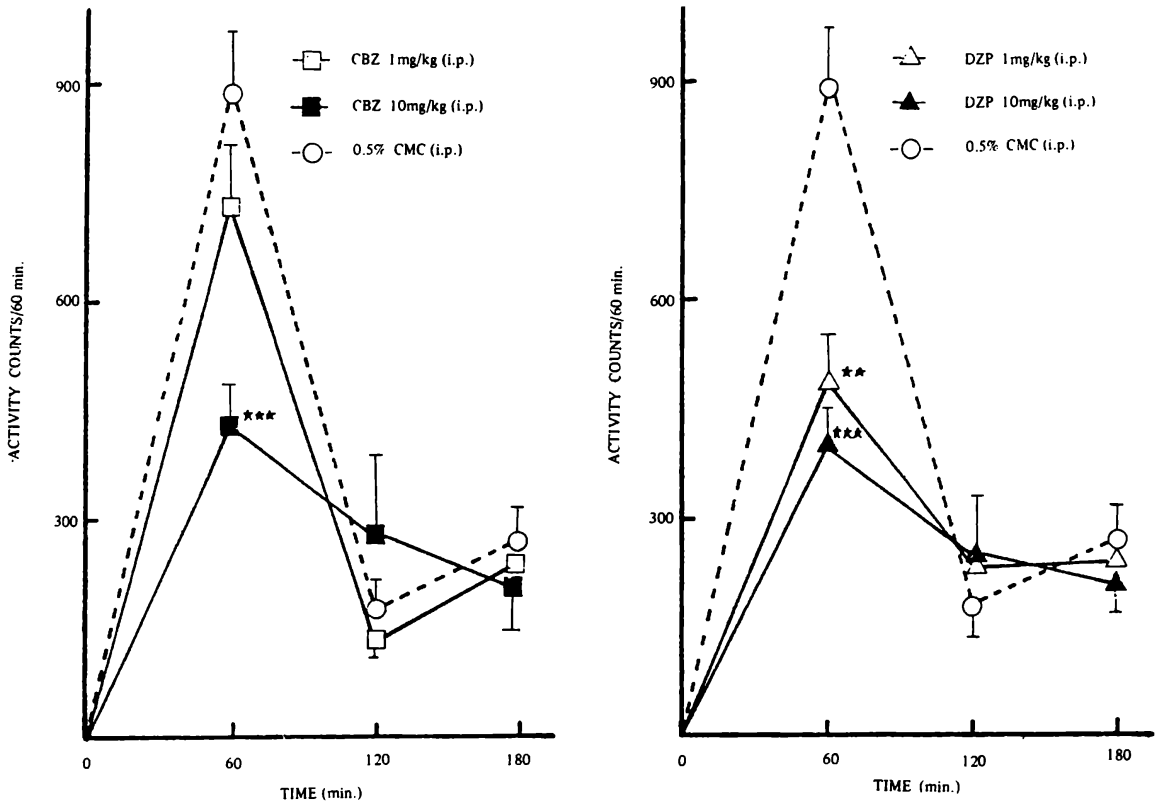


Fig. 2 Effects of clobazam and diazepam on locomotor activity. Rats were injected i.p. with either CBZ or DZP. Locomotor activity was measured at 60-min intervals, for 3 hr, starting 5 min after inserting the rat into the test cage as described in "Methods". In all experiments, each point represents the mean obtained from 10 animals except for the 0.5% CMC group (24 animals). Vertical bars indicate S.E.M. The asterisks indicate values which are significantly different from the corresponding results for control (**P<0.01, ***P<0.001).

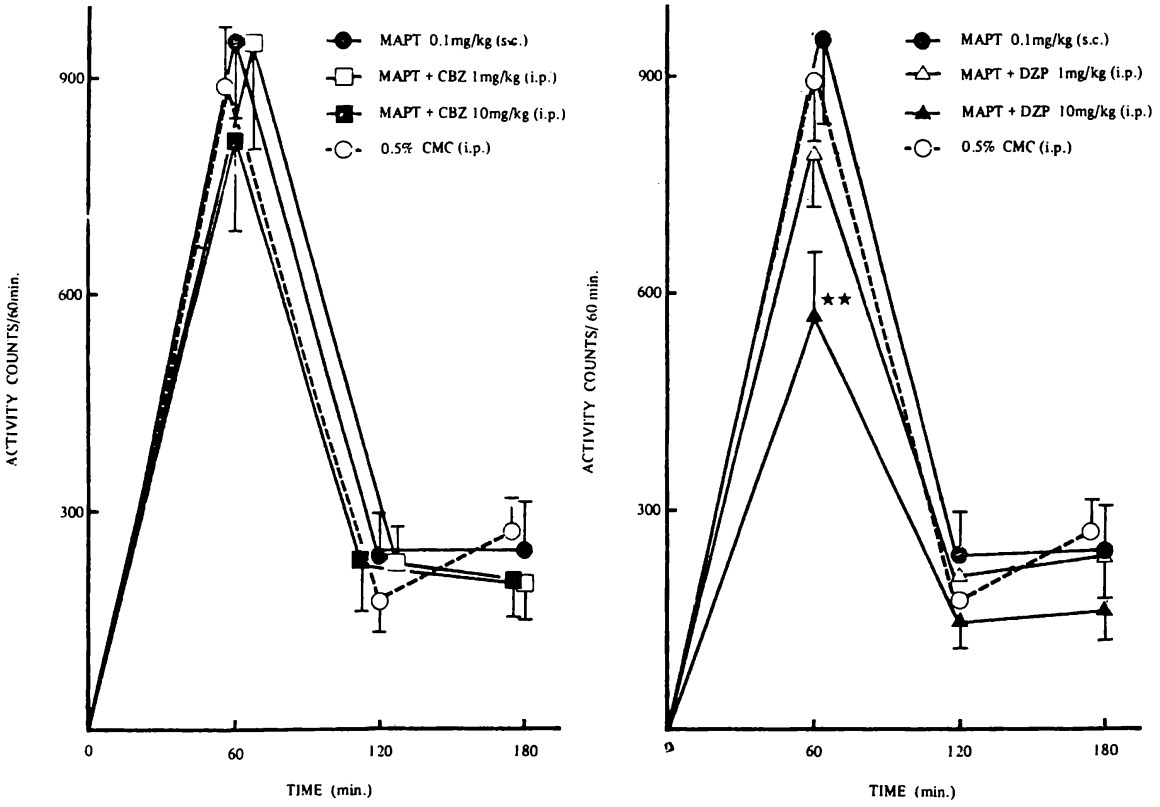


Fig. 3 Effects of CBZ and DZP on changes in locomotor activity produced by MAPT 0.1 mg/kg. An intraperitoneal injection of CBZ or DZP was given simultaneously with MAPT 0.1 mg/kg s.c. For details, see legend for Fig. 2. The asterisks indicate values which are significantly different from the corresponding results for MAPT 0.1 mg/kg administration (**P<0.01).

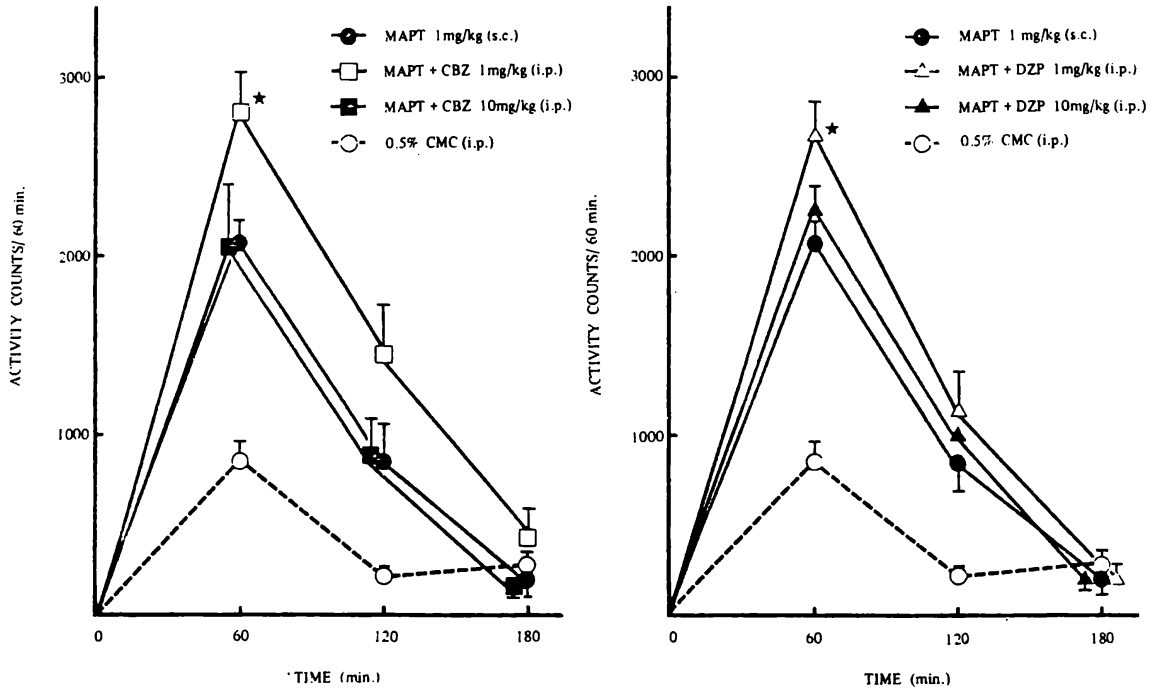


Fig. 4 Effects of CBZ and DZP on changes in locomotor activity produced by MAPT 1 mg/kg. An intraperitoneal injection of CBZ or DZP was given simultaneously with MAPT 1 mg/kg s.c. For details, see legend for Fig. 2. The asterisks indicate values which are significantly different from the corresponding results for MAPT 1 mg/kg administration (*P<0.05)

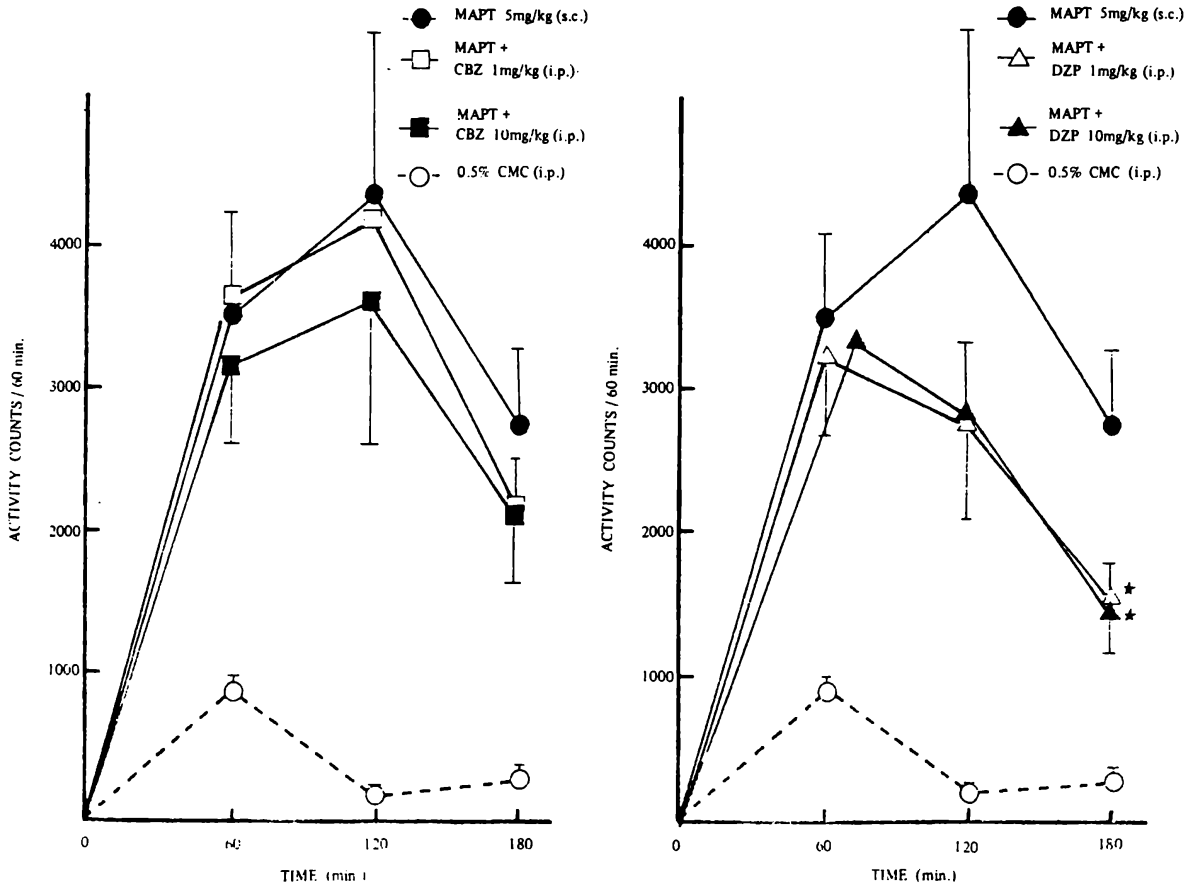


Fig. 5 Effects of CBZ and DZP on changes in locomotor activity produced by MAPT 5 mg/kg. An intraperitoneal injection of CBZ or DZP was given simultaneously with MAPT 5 mg/kg s.c. For details, see legend for Fig. 2. The asterisks indicate values which are significantly different from the corresponding results for MAPT 5 mg/kg administration (*P<0.05)

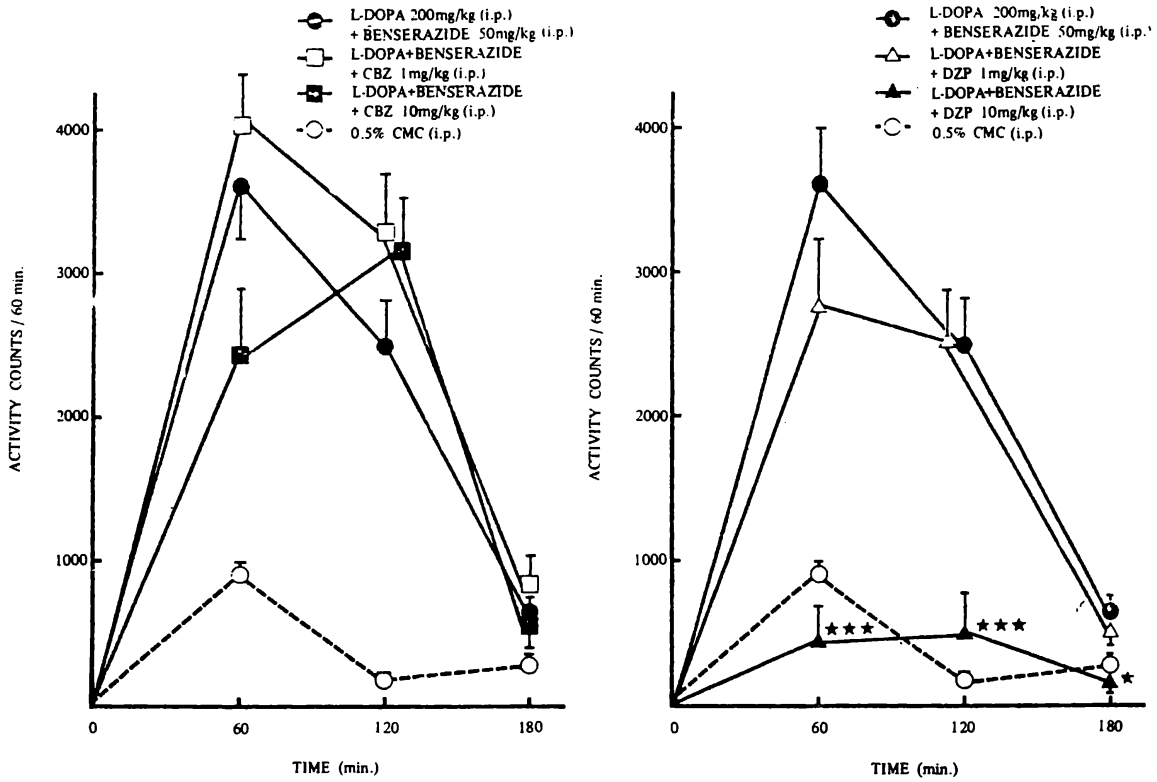


Fig. 6 Effects of CBZ and DZP on changes in locomotor activity produced by L-DOPA plus benserazide. Benserazide 50 mg/kg was injected 80 min and L-DOPA 200 mg/kg 50min before CBZ or DZP. All drugs were injected i.p. For details, see legend for Fig. 2. The asterisks indicate values which are significantly different from the corresponding results for L-DOPA plus benserazide administration (*P<0.05, ***P<0.001)

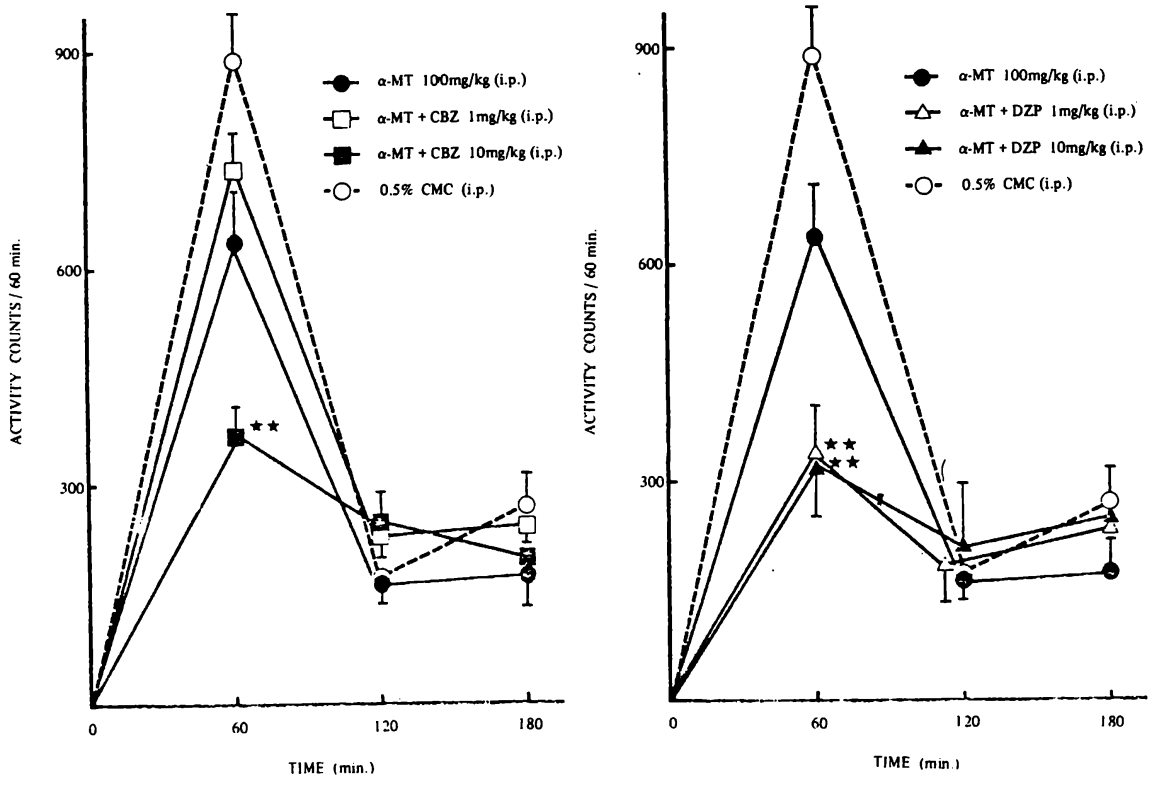


Fig. 7 Effects of CBZ and DZP on changes in locomotor activity produced by α-MT. α-MT was given in a dose of 100 mg/kg 2 hr before CBZ or DZP. All drugs were injected i.p. For details, see legend for Fig. 2. The asterisks indicate values which are significantly different from the corresponding results for α-MT administration (**P<0.01)

activity ($p<0.001$). In contrast to clobazam, diazepam 1 and 10 mg/kg both significantly decreased locomotor activity ($p<0.01$) and ($p<0.001$), respectively. Sedative behavior and decreased ambulation were also seen.

2. The effects of clobazam and diazepam on methamphetamine-produced locomotor activity

(1). The results are shown in Figure 3, MAPT alone in a dose of 0.1 mg/kg produced an increase in activity, but the degree was not significantly different from that with 0.5% CMC ($p>0.1$). The locomotor activity produced by MAPT 0.1 mg/kg alone was markedly reduced by concomitant administration of diazepam 10 mg/kg ($p<0.01$), but not by clobazam 1 and 10 mg/kg.

(2). The results are shown in Figure 4, MAPT alone in a dose of 1 mg/kg caused an increase in activity with peak effects at about 30 min. and a duration of 1-2 hr ($p<0.001$). The locomotor activity produced by MAPT 1 mg/kg alone was significantly potentiated by concomitant administration of clobazam or diazepam 1 mg/kg during the first 60 min. session ($p<0.05$), but not by clobazam or diazepam 10 mg/kg.

(3). Figure 5 shows that rats treated with MAPT 5 mg/kg alone displayed not only a significant increase in locomotor activity ($p<0.001$) between 60 and 180 min., but also a stereotyped sniffing and searching movements were also seen.

The locomotor activity produced by MAPT 5 mg/kg alone was markedly reduced by concomitant administration of diazepam 1 and 10 mg/kg at the 180 min. session ($p<0.05$), but not by clobazam

3. The effects of clobazam and diazepam on L-DOPA plus benserazide-produced locomotor stimulation

The combination of L-DOPA and benserazide caused a greater increase in locomotor activity ($p<0.05$ to $p<0.001$) (Fig. 6) commencing about 30 min after injection. In addition to this locomotor stimulation, stereotyped jumping, head shaking, forepaw padding, sniffing, and licking were observed.

As shown in Figure 6, clobazam 10 mg/kg and diazepam 1 mg/kg slightly but not significantly decreased the response, but locomotor stimulation was strongly reduced by diazepam 10 mg/kg ($p<0.001$) at 60 to 180 min.

4. The effects of clobazam and diazepam on α-MT-produced hypomotility

Rats pretreated with α-MT appeared to be behaviorally depressed ($p<0.1$) was compared to the 0.5% CMC control (Fig. 7). As shown in Fig. 7, the hypomotility produced by α-MT was not affected by clobazam 1 mg/kg, but markedly augmented by clobazam 10 mg/kg, and diazepam 1 and 10 mg/kg ($p<0.01$), as compared to α-MT alone.

DISCUSSION

An acute administration of clobazam has been reported to result in a dose-dependent decrease in locomotor activity in mice[5]. Our present study similarly indicated that single injections of clobazam and diazepam can produce locomotor depression, but clobazam was much less effective

than diazepam. On the other hand, Rastogi et al. found that clobazam produced no significant behavioral change in the rat[8]. The difference between our data and theirs may be due to several kinds of factors, including the apparatus used, route of administration, and other experimental conditions. Moreover, there is the possibility that the CNS mechanisms involved in locomotor depression by clobazam differ from those with diazepam. Therefore, the present study was carried out to differentiate the involvement of central catecholaminergic system in the effects of clobazam and diazepam on motor performance in the rat.

This study indicated that clobazam differs from diazepam in catecholaminergic activity. MAPT, known to exert central stimulant effects, may act via catecholamine release from nerve terminals[9,10]. In our study diazepam specifically suppressed increased locomotor activity induced by MAPT 0.1 and 5 mg/kg, but such a response was not influenced by clobazam. In addition, locomotor stimulation occurred, similar to other studies by administration of L-DOPA plus benserazide[11,12], and was significantly attenuated by diazepam but not affected by clobazam. Moreover, slight hypomotility induced by α-MT, an inhibitor of tyrosine hydroxylase[13,14], was markedly augmented by both clobazam and diazepam.

The present study also shows that MAPT (1 mg/kg)-induced stimulation was significantly enhanced by clobazam or diazepam 1 mg/kg. Our data in consistent with finding of some investigators about amphetamine-induced exploratory activity or stereotyped behavior can be enhanced by benzodiazepines within a suitable dose-ratio[15,16]. Moreover, benzodiazepines significantly increased the concentration of dopamine in the striatal region[8,17], amphetamine-induced stereotyped behavior also is considered to result from the activation of dopaminergic receptors in the striatum[18]. Therefore, we support that the increased dopaminergic activity in the striatum induced by benzodiazepine may explain the increased motor activity by the benzodiazepine-amphetamine combination[19]. But this hypothesis requires further work for confirmation.

Our data suggest that acute administration of clobazam or diazepam may disturb catecholamine function, and, furthermore, that diazepam has a more pronounced effect than clobazam on catecholaminergic neurons where it probably acts antagonistically.

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中 文 摘 要

Clobazam 及 diazepam 對中樞兒茶胺之影響

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本研究爲探討 clobazam (1,5-benzodiazepine) 與 diazepam 對大白鼠行爲運動量之影響，以進行其評價。Diazepam 抑制運動量比 clobazam 强；由 L-DOPA 加 benserazide，及 methamphetamine 所引起自發運動量增强作用被 diazepam 所抑制，但不被 clobazam 所抑制；由 α-MT 所引起運動量抑制作用，二者均能加强。因此 benzodiazepines 之行爲抑制作用可能與減低兒茶胺之活性有關，且 diazepam 減低兒茶胺之活性則比 clobazam 强。